Electronic Supplementary Material (ESI) for Metallomics.

Supplemental Support

Nonspecific interference of cobalt with siderophore-dependent iron uptake pathways

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Promotor regions	Sequences	Gene localisation
PchR box	5'GACAAAGCGCCCTGCACTCCGCCCCTGCAG CGAATGAAAAAGCCCCGCAATCGAAAGGCGC GGGCTTGCGCGGT3'	4742278 -
Fur box	5'CCCTGCCGCCCGCCAATGATAATAAATCTCA TTTCCCAACAATGGCAATCGACCGCATCCACG GAGATCGCATG 3'	4742426 [+]
Fur box	5'GCCGCCCGCCAATGATAATAAATCTCATTTC CCAACAATGGCAATCGACCGCATCCACGGAG ATCGCATG 3'	4742356 - 4742426 [+]

Table 1SM. ***pchD* promoter sequences introduced into pAYC5 vector for the construction of expression plasmids carrying mCherry.** See 'Material and methods'. Red sequence correspond to the PchR box, and blue sequences to the Fur box. The genome location in PAO1 is according to www.pseudomonasaeruginosa.com.

Oligonucleotides	Sequences (5’ to 3’)	Used to construct the following plasmids or in RT-qPCR
pchD prom F	CAAAGAATTCGACAAAGCGCCCTGCACTCCG	pAYC5
pchD prom R	CATGTTATCCTCCTCGCCCTTGCTCACCATGCGATCTCCGTGGATGCG GTGAGCAAGGGCG*AG*GAGGATAACATG	
mcherry F	CTCCAAGCTTTTACTTGTACAGCTCGTCCATGCCGC	
mcherry R		
MutFur F	TAATAAATCT*gtaa*TCCCAACAATGGCAATCGACCGCTGCACCGCATCC ACGGAG	pAYC5-FURmut
MutFur R	TCATTGGCGGGCGGCAGG	
uvrD F	CTACGGTAGCGAGACCTACAACAA	RT-qPCR
uvrD R	GCGGCTGACGGTATTGGA	RT-qPCR
pchA F	CGCGAAACCTGCCTTAAGC	RT-qPCR
pchA R	GTCCAGGCCGCCTATGG	RT-qPCR
pchE F	GGCAATGGCAAGGTCGAT	RT-qPCR
pchE R	CACCGGGCGTTTGAGAAC	RT-qPCR
pvdS F	CAGGCGCTCGAACAGAAATA	RT-qPCR
pvdS R	CGTAGTTGATGTGCGAGGTT	RT-qPCR
pvdJ F	CGTGGCCGCGATATGG	RT-qPCR
pvdJ R	CTCTTCAGGCTGACTTCGATACC	RT-qPCR
fptA F	CGTGGCCGCGATATGG	RT-qPCR
fptA R	CTCTTCAGGCTGACTTCGATACC	RT-qPCR
fptX F	CCCTGGGTGGTCAAGTTCCT	RT-qPCR
fptX R	CGGCGCGACCAGTGA	RT-qPCR
pchR R	GCGCCTGGGCTACAAGATC	RT-qPCR
pchR F	CCGTAGCGGTTGTTCCAGTT	RT-qPCR

Table 2SM: Oligonucleotides used in this study. The mutated residues in pAYC5-FURmut vector are in non-capital letters.

	$FeCl_3$	$CoCl_2$	$NiCl_2$	$ZnCl_2$
pchR	0.06645985	0.954978494015707	1.49665264	1.98795531
	0.06252901	0.957584025403966	1.25575259	0.08943202
	0.05072588	0.965450446181038	0.55587927	0.97924892
Mean	0.05990491	0.95933766	1.1027615	1.01887875
pchA	0.04346023	0.15771836	2.20764985	2.18134132
	0.04409012	0.12956486	0.68718696	0.26671069
	0.03869765	0.12376815	0.72774352	1.11181292
Mean	0.04208267	0.13701712	1.20752678	1.18662164
pchE	0.02923664	0.11095663	3.194277	3.97225907
	0.02285894	0.16083733	1.30681679	2.8159316
	0.02354194	0.12079607	1.79714278	2.8159316
Mean	0.02521251	0.13086334	2.09941219	3.20137409
fptX	0.03483301	0.12469778	1.90421623	3.109841
	0.02094053	0.14288368	0.83928485	0.31812839
	0.01999315	0.11268389	1.00482262	1.95459969
Mean	0.02525556	0.12675512	1.24944123	1.7941897
fptA	0.02091847	0.1345655	4.21045052	5.19471528
	0.01371907	0.12324825	1.04553504	0.49657243
	0.01028649	0.09012936	1.62044297	1.82430115
Mean	0.01497468	0.11598104	2.29214284	2.50519629
pvdS	0.01311133	0.68277492	8.2598626	5.49103089
	0.00995599	0.51011365	1.98679923	1.10294092
	0.00636272	0.40394858	9.59973623	3.37346338
Mean	0.00981001	0.53227905	6.61546602	3.32247839
pvdJ	0.01700111	0.76043686	1.53870177	2.04157712
	0.0082309	0.62422786	0.39201924	0.1898839
	0.008663	0.21540189	0.59857482	0.62539424
Mean	0.01129834	0.53335554	0.84309861	0.95228508

Table 3SM: Experimental data of RT-qPCR experiment presented in Figure 3A.

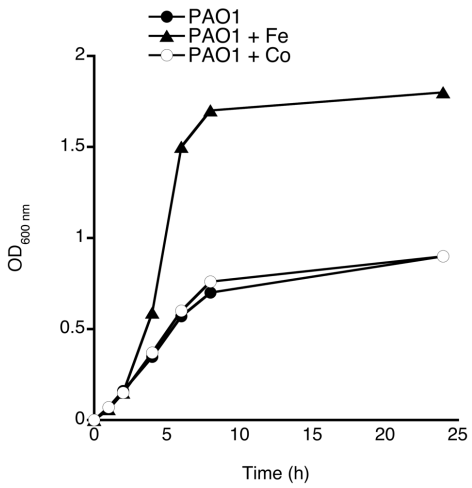

Figure 1SM: Growth curve of PAO1 grown in CAA medium in the absence and presence of 10 µM Fe^{3+} or Co^{2+}. Growth conditions used for proteomic and transcriptomic analyses.

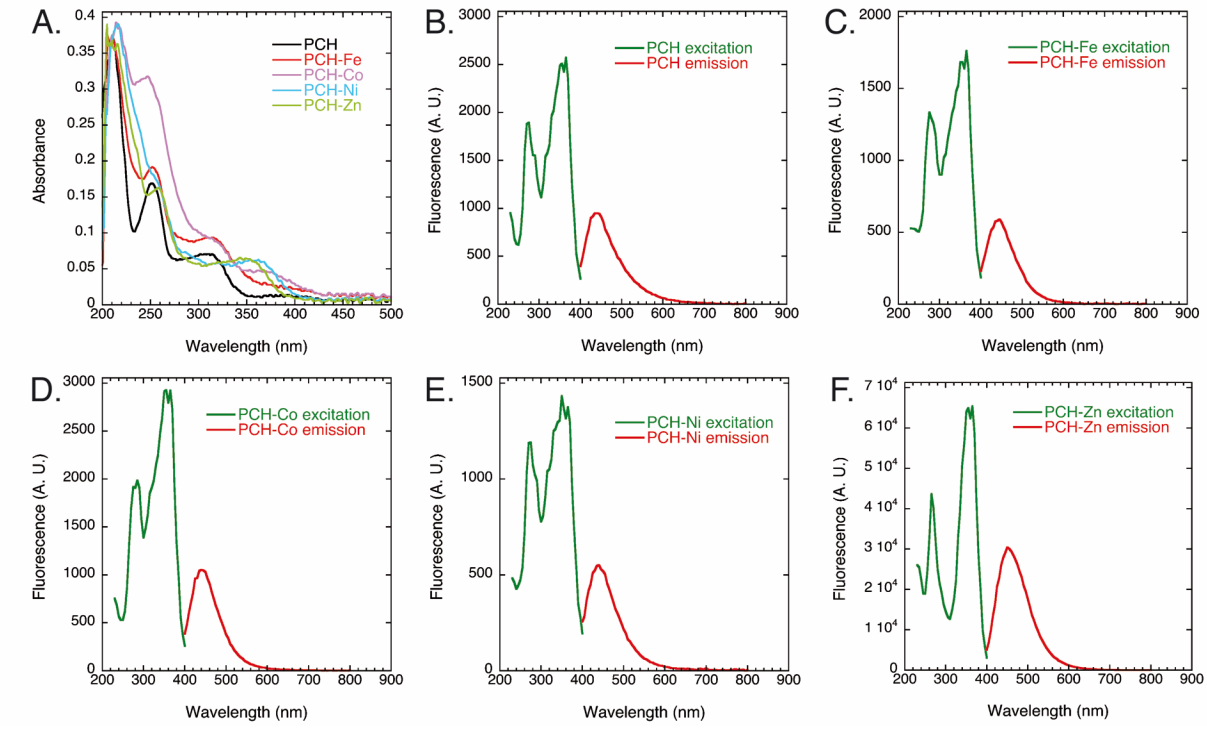

Figure 2SM: A. UV spectra of apo PCH and PCH-metal complexes recorded in 50 mM TrisHCl pH 8.0. **B-F.** Superposition of the normalized emission (red) and excitation (green) spectra recorded in 50 mM TrisHCl pH 8.0 for apo PCH and the different PCH-metal complexes. Emission spectra : λ_{exc} = 350 nm; excitation spectra : λ_{em} = 422 nm.

The complexes have been prepared as described in Materials and Methods by mixing 1 equivalent of metal with 2 equivalents of PCH. UV and fluorescence spectra of apo PCH were carried out at 100 µM for apo PCH and at 50 µM for the PCH-metal complexes. The fluorescence emission scale is not the same in panels B-F: PCH-Zn is much more fluorescent than apo PCH and the other PCH-metal complexes; for PCH-Fe and PCH-Ni the fluorescence is slightly quenched. Apo PCH and PCH-Ni have both a maximum of fluorescence at 438 nm, PCH-Fe at 443 nm, PCH-Co at 440 and PCH-Zn at 452 nm.

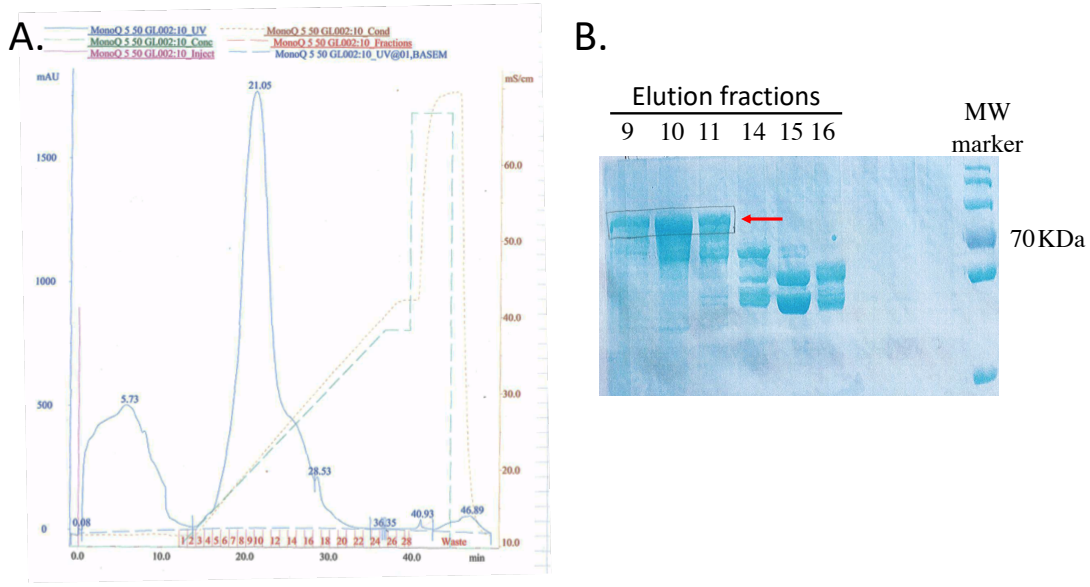

Figure 3SM: MBP-PchR purification. For detailed protocol see in Materials and Methods. **A.** Ionic exchange chromatogram of MBP-PchR (Mono Q™ 5/50 GL). MBP-PchR was eluted with a $NaCl_2$ gradient. **B.** SDS-PAGE analysis of the elution peak at 21 min. MBP-PchR has a molecular weight of 75 kDa and was detected in fractions 9-11.

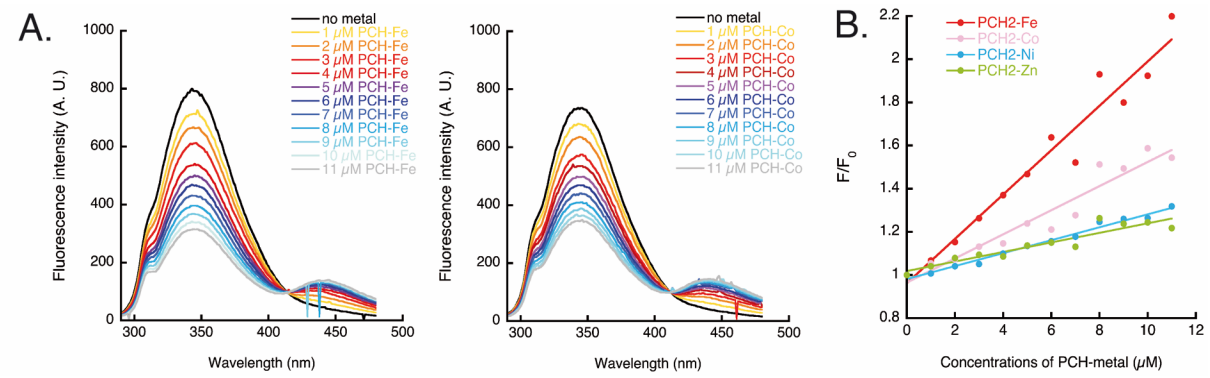

Figure 4SM: A. Fluorescence emission spectra of MBP-PchR in the presence of different PCH-Fe or PCH-Co concentrations. MBP-PchR has a maximal fluorescence emission at 343 nm (black line, excitation at 280 nm). Its fluorescence was quenched with addition of increasing concentrations of PCH-Fe or PCH-Co. PCH-Fe and PCH-Co have a maximal fluorescence emission at 440 nm.
B. Stern-Volmer plot for siderophore binding affinity determination of MBP-PchR. Relative fluorescence intensity (F_0/F) of MBP-PchR at 343 nm in the presence of PCH-Fe, PCH-Co, PCH-Ni and PCH-Zn were plotted against PCH-metal concentrations. Data were fit with a linear regression.

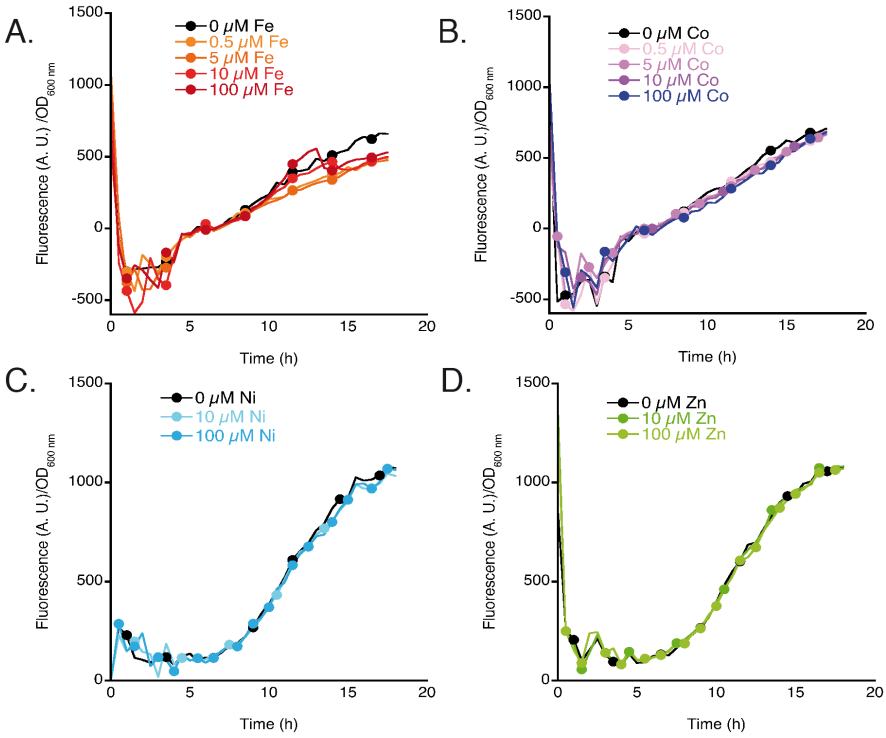

Figure 5SM: Expression of mCherry from pAYC5-FURmut plasmid in the absence or presence of Fe^{3+}, Co^{2+}, Ni^{2+} or Zn^{2+} under Fur regulation in the Δ*pchR* mutant. Δ*pchR* cells were transformed with pAYC5-FURmut plasmid carrying the *pchD* promoter sequence with the PchR box and a Fur mutated box. These cells were grown in CAA medium as PAO1 cells in Figure 7 in the presence of increasing concentrations of Fe^{3+}, Co^{2+}, Ni^{2+} or Zn^{2+}. Bacterial growth was followed at $OD_{600\ nm}$ and mCherry expression by monitoring the emission of fluorescence at 610 nm (excitation at 570 nm). All kinetics are an average of 5 kinetics.